Amendment

In the Specification

NE

On page 37, line 29, before the semicolon, insert

--who states, "The linear sequence of amino acids in the protein has the interesting property that it uniquely determines how [the] chain will fold. The sequence, therefore, controls both the protein's three-dimensional structure and ultimately its function, which depends largely on the protein's shape....Knowing what this three-dimensional structure looks like is crucial to the process of designing drugs....Two spectroscopic techniques help to determine the complex 3-D structures of proteins. The first is X-ray crystallography....A second, and relatively new technique, uses nuclear magnetic resonance (NMR) to detect which atoms of the protein are brought close together by the folding process. By combining these analytical data with building models, we can arrive at a 3-D structure for the protein....[Another idea] is to use the 3-D structures already determined by X-ray crystallography and try to develop rules based on how these proteins fold....To generate [a computer] graphics display, we feed the coordinates or positions of the atoms of the protein structure produced by X-ray, NMR or model building into [a graphics] computer program. At this juncture, the medicinal chemist and molecular modelers are at last ready to begin the process of drug design by computer....The first and most obvious [factor to consider] is that the small molecule must have a geometrically complementary shape to the enzyme's active site (the key must have an appropriate shape to fit the lock). In addition, it must consist of appropriate atoms so that the electronic characteristics of the molecule complements those of the atoms making up the

walls of the active site. In other words, positively charged, polarised, groups of atoms in the

small molecule must fit negatively charged groups in the site, not positively charged groups,

which would repel each other. We are just beginning to understand the rules governing the

relative importance of specific interactions. Finally, the compound must be stable (stay

around long enough to bind to the protein) and relatively easy to synthesize to be a viable

proposition."--.

On page 37, line 30, before the semicolon, insert

--who state, "Rational [antiviral] drug design, defined as the directed synthesis of new

compounds based on an understanding of a prototype drug/viral structural or functional

protein interaction at the atomic level, is only now becoming a viable alternative to empirical

screening....The most important ingredient in a drug design program is the obvious need to

predict the activity of a molecule before the effort is invested to synthesize it. While a

certain amount of success can be had by viewing a ligand/protein structure on a graphics

terminal, the multiplicity of possible conformations the ligand and protein can assume make

it exceedingly difficult to make accurate predictions with any regularity. Recent

developments in the use of thermodynamic calculations and molecular dynamics simulations

permit consideration of previously impossible computational problems through the use of

supercomputers and new computational approaches. The thermodynamic-cyclic perturbation

approach to thermodynamic calculations is likely to be increasingly used in the future

(McCammon, J.A. 1987, Computer-aided molecular design. *Science* 238:486-91.) Since the

free energy change of binding of a drug to a protein or virus would be exceedingly difficult,

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if not impossible, to calculate, this method enables one to calculate the relative free energy change for the binding of two different compounds. These calculations will enable the researcher to predict whether a compound proposed for synthesis is likely to possess a greater binding affinity to the target. The second promising application of computational chemistry to drug design is molecular dynamics simulation (McCammon, J.A., Harvey, S.C. 1987, *Dynamics of Proteins and Nucleic Acids.* Cambridge, London, New York: Cambridge Univ. Press, pp. 234). The object of this exercise is to simulate the dynamics of the drug/protein interactions based on the dynamical trajectories of each atom. This type of analysis can help identify areas of the drug where considerable movement is occurring when bound to the target, suggesting that the conformation of the drug may need to be constrained to maximize activity. This analysis may also suggest ways in which the drug is exerting its effect on the protein."--.

On page 37, line 32, before the semicolon, insert

--who state, "**ChemStat** has been developed to allow the rapid construction of a database containing molecular coordinates together with computed and experimental parameters and biological data....The database is stored on disk and comprises a number of segments. Each segment is a molecular structure together with associated information stored in 104 data fields. Some or all of the segments may be read into the **Chem-X** data matrix where information may be added, deleted or modified. Data may be placed in the fields by calculating properties within **Chem-X** (or interfaced programs) or by reading from an external file. Each property to be calculated must first be defined in terms of a **ChemStat**

template file. This flexible method allows definitions from the supplied 'library' of

calculation types, user-supplied **Chem-X** command files or external programs interfaced

through **ChemLib**. An automatic procedure is then invoked whereby each property is

calculated for each segment before the database is updated. This will generally result in a

data matrix containing a vast amount of information which must be 'sifted' before it can be

used predictively. Key fields must be identified as being associated with the required

property. A novel method of data reduction is employed by **ChemStat** to address this

problem. One field in the data matrix is specified as being the 'observed' field (generally the

biological activity). By the automatic calculation of the appropriate correlation coefficients,

those fields which correlate best with this field but are not highly inter-correlated can be

listed and passed to the reduced data matrix. The data may be viewed graphically or in

tabular form and fields and segments may be interactively included or excluded from the

calculations. More sophisticated statistical techniques such as principal component analysis

or pattern recognition are made available through an interface to some of the more popular

commercial packages. Data from the reduced data matrix is passed to the program and new

or modified data can be passed back to **Chem-X** via an intermediate file. Once the key

fields have been identified, they can be used as predictor variables for multiple regression

against the 'observed' field. **ChemStat** will determine the regression equation and calculate

predicted values for the 'observed' variable from the values of the predictor variables for

each segment....ChemStat, ChemLib and ChemQH are modules of the molecular modelling

software Chem-X, developed and distributed by Chemical Design Limited, Oxford,

England."--.

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On page 37, line 33, before the semicolon, insert

--who state, "This paper examines the problem of automated structure generation at specified

binding sites. The objective is to obtain molecular graphs that span the binding site and

incorporate predicted ligand points at their vertices. Three approaches are considered: brute

force techniques, subgraph addition and spacer skeletons. A spacer skeletons is a device for

representing several distinct classes of compound within just one structure. It is used to

reduce the amount of searching that must be done in the primary phase of structure

generation....The CDS [Cambridge Structural Database] can be searched to find all the

compounds that contain a certain molecular fragment and the geometric parameters of this

fragment in each compound can be calculated. Certain geometric calculations can be

performed directly on the DATA file at the retrieval stage and the redundant information on

irrelevant parts of the molecule is ignored. The GEOM program will compute angles,

centroids, vectors, planes and internal coordinates for a user-specified fragment (Allen *et al.*

1979 [*Acta crystallogr.* B 35,2331-2339]). This powerful facility can provide the user with a

comprehensive range of geometric parameters, which can then be analysed statistically. This

database is of great use in the building of spacer skeletons designed to model many different

fragments....The results of each search through the database were read into a geometric

analysis program which retrieved the coordinate data from the DATA file, plotted out the

whole molecule by using the PLUTO 78 package (Motherwell & Clegg 1978 [PLUTO78.

Program for plotting molecular and crystal structures. In *Cambridge crystallographic files*.

University of Manchester Regional Computer Centre.]) and calculated the least-squares plane

through the atoms in the fragments. The deviation of each atom from the plane was

computed together with the distance from the atom to the centroid of the fragment. The

results were plotted as frequency histograms of (i) the root mean square deviation and (ii) the

distance of highly deviant atoms from the centroid....The program MNET was written to

provide a reliable means of constructing spacer skeletons either round a starting nucleus or

from scratch."--.

On page 37, line 35, before the period, insert

--who state, "The most relevant precedent for base pairing in model systems is that of Rich,

[Kyogoku et al., *J. Am. Chem. Soc.* 1968, 90,4151-4157,] using cyclohexyluracil binding to

9-ethyladenine in $CHCl_3$. Systematic structural modifications in both components were made

in this study and revealed trends concerning steric effects and acid-base effects. At the same

time these systems were used to examiner the kinetics of the base-pairing event. Aromatic

stacking interactions of simple bases were studied by Chan [Chan et al., *Am. Chem. Soc.*

1964, 86, 4182. Schweitzer et al. *Ibid.* 1965, 87, 5241-5247. Iwahashi et al. *Ibid.* 1977, 99,

7761-7765.] in aqueous solutions, whereas Tinoco et al. [Tinoco et al., *Nature, New Biol.*

1973, 246, 40-41] have developed a set of rules for the sequence-specific hydrogen bonding

and stacking contributions of various base pairs to the stability of intact nucleic acids. Our

departure from previous model studies is made possible by the construction of a new

molecular shape which permits both hydrogen bonding and aromatic stacking forces to act

simultaneously. The structural developments are a consequence of the use of Kemp's [Kemp

et al., J. Org. Chem. 1981, 46, 5140-5143] triacid **3**, in which a U-shaped (diaxial)

relationship exists between any two carboxyl functions. Conversion of the triacid to the

imide acid chloride **4b** gives an acylating agent that can be attached via amide or ester

linkages to practically any available aromatic surface. The resulting structure features an

aromatic plane which can be roughly parallel to that of the atoms in the imide function;

hydrogen bonding and stacking forces converge from perpendicular directions to provide a

microenvironment complimentary to adenine derivatives. The same structural features are

also present in a model for thymine and uracil recognition developed by Hamilton [Hamilton

et al., J. Am. Chem. Soc. 1987, 109, 5035-5036.]"--.

On page 39, lines 27-28, please delete, without prejudice, the words "are specifically

incorporated by reference. Other" and insert therein the words --and other--.

On page 40, delete lines 1-2.

In the Claims

1. (thrice amended) A method for designing [compounds] a compound

specifically inhibiting targeted ribonucleic acid function comprising the steps of:

(a) determining the nucleotide sequence in the targeted ribonucleic acid that is

critical to function;

(b) determining the secondary structure of the region of the targeted ribonucleic

acid in which the critical site is located;